An internet-based approach for lifestyle changes in patients with NAFLD: Two-year effects on weight loss and surrogate markers

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Author links open overlay panelAriannaMazzotti[1]Maria TurcheseCaletti[1]LuciaBrodosi[1]SilviaDi Domizio[1]Maria LuisaForchielli[1]SalvatorePetta[2]ElisabettaBugianesi[3]GiampaoloBianchi[1]GiulioMarchesini[1]

Highlights

•Job/time constraints limit the engagement of patients with NAFLD in counseling programs.
•Web- and group-based programs promote similar calorie/physical activity changes.
•Surrogate markers indicate reduced fat in the liver and no changes in hepatic fibrosis.
•Web counseling results in clinically significant weight loss in motivated patients.
•Structured web-based program is as effective as group-counseling in selected patients with NAFLD.

Background & Aims. Interventions aimed at lifestyle changes are pivotal for the treatment of non-alcoholic fatty liver disease (NAFLD), and web-based programs might help remove barriers in both patients and therapists.

Methods. In the period 2010–15, 716 consecutive NAFLD cases (mean age, 52; type 2 diabetes, 33%) were treated in our Department with structured programs. The usual protocol included motivational interviewing and a group-based intervention (GBI), chaired by physicians, dietitians and psychologists (five weekly meetings, n = 438). Individuals who could not attend GBI entered a web-based intervention (WBI, n = 278) derived from GBI, with interactive games, learning tests, motivational tests, and mail contacts with the center. The primary outcome was weight loss ≥10%; secondary outcomes were alanine aminotransferase within normal limits, changes in lifestyle, weight, alanine aminotransferase, and surrogate markers of steatosis and fibrosis.

Results. GBI and WBI cohorts had similar body mass index (mean, 33 kg/m^2), with more males (67% *vs.* 45%), younger age, higher education, and more physical activity in the WBI group. The two-year attrition rate was higher in the WBI group. Healthy lifestyle changes were observed in both groups and body mass index decreased by almost two points; the 10% weight target was reached in 20% of WBI cases *vs.* 15% in GBI (not significant). In logistic regression analysis, after adjustment for confounders and attrition rates, WBI was not associated with a reduction of patients reaching short- and long-term 10% weight targets. Liver enzymes decreased in both groups, and normalized more frequently in WBI. Fatty liver index was reduced, whereas fibrosis remained stable (NAFLD fibrosis score) or similarly decreased (Fib-4).

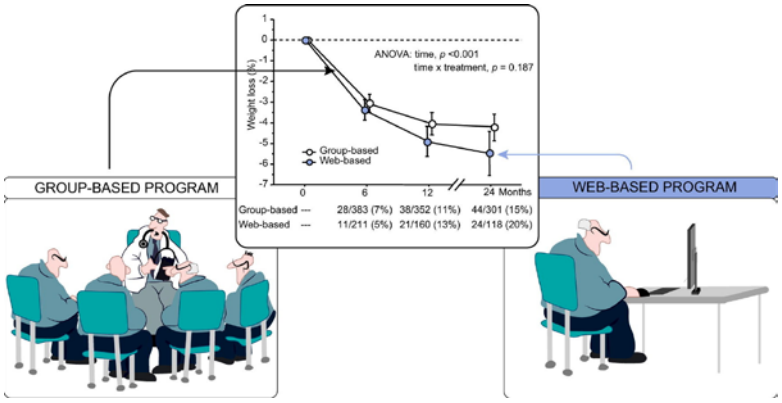
Conclusion. WBI is not less effective than common lifestyle programs, as measured by significant clinical outcomes associated with improved histological outcomes in NAFLD. eHealth programs may effectively contribute to NAFLD control.

Lay summary

In patients with non-alcoholic fatty liver disease, participation in structured lifestyle programs may be jeopardized by job and time constraints. A web-based intervention may be better suited for young, busy patients, and for those living far from liver units. The study shows that, following a structured motivational approach, a web-based, interactive intervention coupled with six-month face-to-face meetings is not inferior to a standard group-based intervention with respect to weight

loss, adherence to healthy diet and habitual physical activity, normalization of liver enzymes, and stable surrogate markers of fibrosis.

Graphical abstract



Keywords

Diet
Liver enzymes
NAFLD progression
Physical activity

Introduction

The burden associated with non-alcoholic fatty liver disease (NAFLD) is becoming a major problem for health systems worldwide.[1] As part of the metabolic syndrome, NAFLD prevalence is increasing in parallel with the epidemics of obesity and diabetes;[2] although in most cases NAFLD remains a non-progressive disease, in some cases non-alcoholic steatohepatitis (NASH) and progressive fibrosis may occur, finally progressing to cirrhosis and hepatocellular carcinoma.[3] Thus, the costs associated with liver disease of metabolic origin and its complications are likely to soon outweigh the costs of liver diseases of viral origin.[4]

Several drugs are under investigation to stop NAFLD progression,[5] but none have been approved so far by regulatory agencies. Like any non-communicable disease, lifestyle changes remain the cornerstone of NAFLD prevention and treatment, and are also the background treatment suggested by all clinical practice guidelines, including the recent European guidelines shared by the Liver, Diabetes and Obesity Societies.[6]

Programs to promote lifestyle changes have been developed in the community, mainly in the area of obesity and diabetes, following the seminal Finnish Diabetes Prevention Study and U.S. Diabetes Prevention Program. Their effectiveness in promoting weight loss has been demonstrated on long-term follow-up.[7] Programs of cognitive-behavioral therapy have also been applied in NAFLD;[8] weight loss through healthy and restrictive diet, coupled with habitual physical activity, has been reported to reduce NAFLD progression in small pilot trials,[9-11] and a large prospective intervention study confirmed that weight loss is associated with histologic improvement on repeated liver biopsy.[12] Unfortunately, these programs require dedicated teams and support, which are rarely found in liver units.[13] In addition, it may be difficult to engage asymptomatic, scarcely motivated patients with NAFLD in intensive lifestyle protocols, because of space and time constraints.[14]

eHealth technology is a possible resource to promote behavior changes,[15] thus reducing NAFLD

progression. The possibility to educate, to counsel and to induce permanent changes in motivated and engaged patients with NAFLD via an internet-based approach would reduce attendance to busy liver units, sparing patients' and physicians' time, and would expand lifestyle intervention to a much larger community.[13]
The present study was aimed at measuring the effectiveness of a web-based educational intervention aimed at lifestyle changes, including healthy diet and habitual physical activity, and weight loss in individuals with NAFLD.

Materials and methods

Patients

The study involves individuals with ultrasonography-diagnosed NAFLD attending the Unit of Metabolic Diseases and Clinical Dietetics, University of Bologna, from January 2010 to December 2015. During this period a web-based lifestyle modification program was set up, funded as part of the subproject FP7/2007-2013 FLIP (Fatty Liver – Inhibition to Progression), under grant agreement No. HEALTH-F2-2009-241762. The majority of cases were part of a NAFLD cohort attending our outpatient service, which serves as second-level center for obesity and diabetes. A few were specifically addressed to our center for a second opinion because of NAFLD diagnosed elsewhere. According to our procedures, all NAFLD cases are routinely invited to enter a group-based lifestyle modification program following initial assessment, diagnostic procedures and motivational interviewing.[16] The initial assessment includes routine biochemistry, the measurement of calorie intake by an *in house* developed questionnaire (Quanto Mangio Veramente? – How much do I really eat?)[17], [18] and of habitual physical activity by the International Physical Activity questionnaire.[19] The program, in use in our unit since 2003,[20] was initially devised for patients with obesity and or type 2 diabetes (T2DM), and has been partly adjusted in the course of the years to cover the few specific needs of patients with NAFLD (see below). Patients who agreed to treatment (n = 438) entered and completed the lifestyle modification program within three months (group-based intervention – GBI).
The web program was specifically designed for individuals who could not attend the standard program; these individuals (n = 278) were provided a user-id and a password to access web-based intervention (WBI), largely reproducing the protocol and the tools of GBI. The main reasons for preferring the web program were time or job constraints, preventing attendance during weekdays, or living far from the center; the reasons for favoring the group-based therapy were scarce ability with e-technology and more severe comorbidities, possibly requiring more frequent contact with therapists.
The socio-demographic and clinical data of the entire NAFLD cohort are presented (Table 1). These groups do not include cases (<5%) who received individual, face-to-face education because of specific needs. After enrollment in either program, all patients attended the clinic for follow-up visits every six months, receiving reinforcement and treatment for comorbidities, but no specific therapy for their liver disease. Patients with T2DM received drugs with possible hepatic effects: metformin, 73.1%; pioglitazone, 4.6%; glucagon-like peptide-1 receptor agonists, 3.7%; insulin, 3.2% (all, no differences between WBI and GBI). Surrogate markers of steatosis (Fatty Liver Index – FLI)[21] and fibrosis (NAFLD fibrosis score – NFS,[22] Fibrosis-4 Calculator – Fib-4)[23] were tested at baseline and at 12- and 24-month follow-up.

Table 1. **Socio-demographic, clinical and biochemical characteristics of patients with NAFLD enrolled in the lifestyle intervention program.**

	Total (n = 716)	Web–treated (n = 278)	Group–treated (n = 438)	*p* value*
Sex (Males,%)	53.5 (49.8–57.0)	66.9 (61.0–72.0)	45.0 (40.3–49.5)	<0.001
Age (years)	51.6 ± 12.8	46.0 ± 11.5	55.1 ± 12.3	<0.001
Weight (kg)	94.5 ± 18.7	99.4 ± 20.8	91.4 ± 16.5	<0.001
Height (cm)	168.1 ± 10.4	171.6 ± 10.2	165.9 ± 9.8	<0.001
BMI (kg/m^2)	33.4 ± 5.5	33.7 ± 6.0	33.2 ± 5.2	0.273
Obesity (%)	70.4 (66.9–73.6)	67.6 (61.7–72.7)	72.1 (67.7–76.0)	0.196
Waist circumference (cm)	106.4 ± 12.2	107.6 ± 13.2	105.6 ± 11.5	0.033
Waist ≥102 (M), ≥88 (F) (%)	42.5 (38.8–46.0)	29.8 (24.8–35.3)	50.5 (45.7–55.0)	<0.001
Systolic pressure (mmHg)	132.2 ± 13.7	129.3 ± 13.3	134.0 ± 13.7	<0.001
Diastolic pressure (mmHg)	84.9 ± 9.0	83.5 ± 8.8	85.8 ± 9.0	0.008
Diabetes (%)	33.2 (29.8–36.7)	21.6 (17.0–26.6)	40.6 (36.0–45.2)	<0.001
Prediabetes (IFG/IGT,%)	8.5 (6.6–10.7)	7.2 (4.6–10.6)	9.4 (6.9–12.5)	0.339
Education				
Primary/secondary/vocational/ university (%)	1/23/50/26	1/10/48/41	1/32/51/16	<0.001
Residence				
Within the metropolitan area (%)	58.5 (54.8–62.0)	41.7 (35.9–47.4)	69.2 (64.6–73.2)	<0.001
Employment status (%)				
Student/housewife/employed/self–employed/retired (%)	2/8/60/18/12	3/2/67/25/3	1/12/56/13/18	<0.001
Fasting biochemistry				
Glucose (mg/dl)	112.0 ± 34.0	100.5 ± 25.0	119.6 ± 36.9	<0.001
Insulin (mU/L)	20.1 ± 13.2	20.6 ± 14.8	19.7 ± 11.8	0.410
HOMA-R (%)	5.32 ± 3.68	5.10 ± 3.99	5.48 ± 3.42	0.222
Glycosylated hemoglobin (%)	7.22 ± 3.08	6.23 ± 1.64	7.54 ± 3.35	0.002
AST (mU/ml)	34.9 ± 17.8	31.9 ± 17.4	36.8 ± 17.9	<0.001
ALT (mU/ml)	55.2 ± 32.8	48.8 ± 28.8	59.3 ± 34.5	<0.001
Normal ALT (%)	14.2 (11.8–16.9)	18.3 (14.1–23.1)	11.6 (8.9–14.9)	0.016
GGT (mU/ml)	62.5 ± 59.8	53.3 ± 55.0	57.4 ± 40.4	0.257
Total cholesterol (mg/dl)	212.2 ± 42.5	205.0 ± 42.0	216.8 ± 42.2	<0.001
HDL–cholesterol (mg/dl)	46.4 ± 11.1	45.5 ± 10.6	47.0 ± 11.4	0.070
Triglycerides (mg/dl)	181.7 ± 117.2	165.1 ± 141.8	192.2 ± 97.2	0.003
LDL–cholesterol (mg/dl)	132.3 ± 38.0	129.7 ± 37.5	133.9 ± 38.4	0.150
Surrogate markers				
Fatty liver index (%)	82.3 ± 17.2	81.0 ± 17.5	83.1 ± 17.0	0.107
FLI ≥60% (%)	88.5 (85.9–90.6)	87.8 (83.2–91.0)	89.0 (85.6–91.5)	0.849
Fib-4 score	1.22 ± 0.61	1.00 ± 0.46	1.36 ± 0.64	<0.001

	Total (n = 716)	Web–treated (n = 278)	Group–treated (n = 438)	*p* value*
Fib-4 <1.45 (no severe fibrosis) (%)	72.9 (69.5–76.0)	86.3 (81.6–89.7)	64.4 (59.7–68.6)	<0.001
Fib-4 >3.25 (severe fibrosis) (%)	1.3 (0.6–2.3)	0.4 (0.0–1.7)	1.8 (0.9–3.4)	
NAFLD Fibrosis score	–0.50 ± 1.30	–0.99 ± 1.32	–0.18 ± 1.18	<0.001
NAFLD Fibrosis score <−1.455 (%)	23.5 (20.4–26.6)	36.3 (30.7–42.0)	15.3 (12.1–18.8)	<0.001
NAFLD Fibrosis score >0.676 (%)	17.6 (14.9–20.5)	9.7 (6.6–13.6)	22.6 (18.8–26.6)	

*Chi-square, Fisher's exact, Mann-Whitney or Student's *t* test, as appropriate.

Data are presented as mean ± SD or as prevalence (95% CI).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FLI, fatty liver index; GGT, gamma-glutamyltransferase; HOMA, homeostasis model assessment; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MET, metabolic equivalents; NAFLD, non-alcoholic fatty liver disease.

The WBI and the study were approved by the ethical committee of S. Orsola-Malpighi Hospital, Bologna, as an interventional, non-pharmacologic study, and patients signed an informed consent before entering the program. Comparison with the standard treatment is part of an internal audit to test the effectiveness of WBI on specific outcomes.

Group-based program

The program has been detailed in previous papers.[20,24] Briefly, it consists of group counseling (20–25 persons) on healthy diet, according to the principles of a Mediterranean diet, and on habitual physical activity. It consists of five 120 min weekly sessions, chaired by physicians and dietitians, covering: a) energy balance, nutrients and weight monitoring; b) alimentary pyramid and portion size; c) food shopping, food labels; d) physical activity, when and how much. The final session is chaired by a psychologist and covers the behavioral strategies for stimulus control and weight loss maintenance.

Web-based program

The web program was developed on a Cloud/SaaS e-learning platform by Docebo SpA (Biassono, MB, Italy). It reproduces the group program and is similarly divided into the four sessions detailed above, plus a final role-game measuring adherence to previous learning objectives. All sessions begin with an introductory message, followed by an online questionnaire to investigate consciousness, motivation to change and competence in the specific area. After completing the questionnaire, the patients are provided with a series of 25–35 slides per sessions, with texts read by a voiceover and figures to support the text. Slides are highly interactive and include examples to be completed and games to measure learning objectives. The individual sessions may be repeated without limitations, and patients may interact with the clinical center offline, by sending food diaries or asking questions via specific tools. The system traced the completion of the program, but most interaction occurred via e-mails and was not recorded.

Study outcomes

Considering that all individuals were characterized by overweight/obesity, the primary outcome of intervention was weight loss. The outcome for analysis was set at 10% weight loss, following the evidence that 10% weight loss achieved by intense lifestyle changes improves NAFLD histology.[12] Many secondary outcomes were also tested: a) percentage changes in body mass index (BMI); b) return of alanine aminotransferase (ALT) levels within normal values (defined according to the updated reference values of ≤31 mU/ml in males and ≤19 in females); c) Changes in dietary intake and habitual physical activity (measured at baseline and after six months, to support treatment effectiveness); d) changes in surrogate markers of steatosis (FLI score and number of cases with FLI <60) and fibrosis (scores of Fib-4 and NFS and fibrosis stage).
Very few patients were submitted to liver biopsy in the Bologna center; a few more liver biopsies were available in individuals referred to Bologna for a second opinion, but follow-up biopsies were rarely performed and were not considered as an outcome in the present study.

Statistical analysis

A complete database was constructed merging data from the two groups at different time points (baseline, and 6-, 12- and 24-month follow-up). All anthropometric data were available; missing biochemical and behavioral values (less than 10% for each variable) were imputed using the last-observation-carried-forward technique. Initially, descriptive statistics were created by computing means ± standard deviation for the entire population, as well as for the WBI and GBI cohorts. For nominal data, the prevalence and 95% confidence interval (CI) was calculated. Comparison between groups was carried out by Student's *t* test for unpaired data, chi-square test and Fischer's exact test, as appropriate. Longitudinal changes in clinical data were compared by paired *t* test and repeated ANOVA, considering individuals in active follow-up. Factors associated with primary and secondary outcomes (dependent variable) were tested by logistic regression analysis, with type of intervention and confounders as independent variables. Given the large difference between the two cohort populations, the set of confounders included age, sex, education, employment, baseline BMI and the presence of diabetes. ALT at baseline and changes in BMI were also added as confounders in the analysis of ALT target reach.

Results

Characteristics of the web-treated population

When compared with individuals enrolled in the standard GBI, WBI individuals were characterized by a relative excess of males and younger age (Table 1). Although mean BMI was similar, there was a relative, slightly higher proportion of overweight and a lower prevalence of obesity. Blood pressure was significantly lower, and the prevalence of diabetes was nearly halved. In addition, they had a much higher educational level, and the employment status reflected differences in gender prevalence and age (lower prevalence of housewives and retired individuals).
Biochemistry confirmed lower glucose levels – and lower glycosylated hemoglobin in the presence of diabetes – lower liver enzyme and lipid levels. ALT levels were above the updated reference values (≥31 in males and ≥20 in females) in 74% of males and 97% of females.
FLI was above the cut-off for the diagnosis of steatosis in 88% of cases (not different between groups), whereas Fib-4 indicated that severe fibrosis was rare, with only one case in WBI *vs.* 8 in GBI (p <0.001). NFS excluded and predicted severe fibrosis in 36% and 10% of WBI cases, respectively, *vs.* 15% and 23% of the GBI cohort (p <0.001).
The moderately lower severity of liver disease in the WBI group was generally confirmed in the very few cases submitted to liver biopsy within six months prior to entering the lifestyle programs (Table S1).

Attrition rates

In WBI, the number of cases regularly attending control visits decreased progressively to 76%, 58% and 43% at 6-, 12- and 24-month follow-up. These figures are lower than those observed in GBI (87%, 80% and 69%, respectively; $p < 0.001$). Attrition rates were more common in females, irrespective of groups ($p < 0.001$). After adjustment for age, sex, education and employment status, severity of obesity and presence of T2DM, both short-term (six-month) and long-term attrition rates were significantly associated with WBI (odds ratio (OR) 1.87; 95% CI 1.20–2.90; and OR 2.95; 95% CI 2.04–4.26, respectively). In both short- and long-term analysis, attrition rates were also significantly associated with enzyme levels within normal ranges at baseline (OR 2.39; 95% CI 1.40–4.07, and OR 1.63; 95% CI 1.01–2.64, respectively), and were reduced in the presence of diabetes (OR 0.58; 95% CI 0.36–0.96, and OR 0.46; 95% CI 0.32–0.67).

Lifestyle changes

At baseline the dietary intake was not different in the two cohorts, but WBI individuals derived a moderately higher amount of calories from carbohydrates and a lower amount from lipids (Table 2). The amount of physical activity was generally low, but higher in the WBI cohort.

Table 2. Lifestyle habits at baseline (last one week) and after six months, according to treatment groups **(mean ± SD).**

Variable	Baseline			6 months		
	Web–treated n = 211	**Group–treated n = 383**	***p* value**	**Web–treated n = 160**	**Group–treated n = 352**	***p* value**
Calorie intake (kcal/day)	2,066 ± 455	2,028 ± 327	0.189	1,801 ± 272	1,833 ± 265	0.174
Carbohydrate content (%)	49.8 ± 7.1	48.7 ± 6.8	0.030	–	–	–
Protein content (%)	15.6 ± 2.8	15.2 ± 2.7	0.057	–	–	–
Lipid content (%)	34.4 ± 6.3	36.1 ± 6.2	<0.001	–	–	–
Physical activity (MET/h/wk)	19.5 ± 14.8	15.5 ± 14.4	<0.001	28.6 ± 13.8	23.2 ± 15.7	<0.001

*Significantly different from the corresponding value in group-treated cases (chi-square, Fisher's exact, Mann-Whitney or Student's *t* test, as appropriate).

After six months, calorie intake significantly decreased in the two cohorts, more markedly in WBI (WBI, −273 ± SE 31 kcal/day; GBI, −193 ± 13 in GBI; $p = 0.006$, paired *t* test), whereas physical activity significantly increased from baseline, without differences between groups (WBI, +9.5 ± SE 1.0 MET/hour/week; GBI, +8.1 ± 0.6; $p = 0.183$) (Table 2).

Primary weight loss outcome

In patients compliant to follow-up, BMI decreased progressively in both treatment arms by nearly two points, without differences between groups (time x treatment ANOVA, $p = 0.063$). In the WBI cohort, body weight decreased on average by 3.4% at 6 months, by 4.9% at 12 months and 5.5% at

12 months (all p <0.001, paired t test) in the per protocol analysis, and similarly by 3.1%, 4.0% and 4.2% in the GBI cohort (Fig. 1 and Table 3).

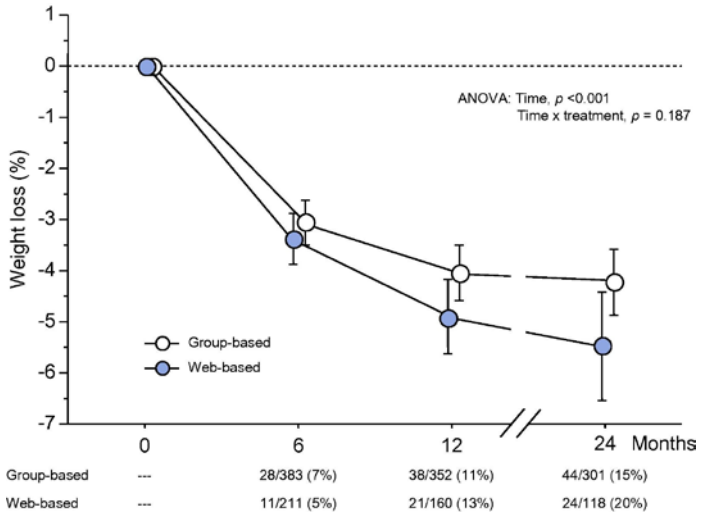


Fig. 1. Percentage weight loss in patients with NAFLD enrolled in the web-based (grey circles) and the group-based (white circles) lifestyle intervention programs. The number of cases achieving the 10% weight loss target at different time points is reported in the Table. Note that no differences between groups were demonstrated at any time point. NAFLD, non-alcoholic fatty liver disease.

Table 3. Weight loss and changes in biochemistry in NAFLD, according to treatment group (mean ± SE).

Variable	6 months		12 months		24 months	
	Web–treated n = 211	Group–treated n = 383	Web–treated n = 160	Group–treated n = 352	Web–treated n = 118	Group–treated n = 301
Δ Body weight (%)	−3.4 ± 0.2*	−3.1 ± 0.2	−4.9 ± 0.4*	−4.0 ± 0.3	−5.5 ± 0.5*	−4.2 ± 0.3
Weight loss >5% (%)	21.3	20.1	28.1	23.9	23.7	25.6
Weight loss >10% (%)	5.2	7.3	13.1	10.8	20.4	14.6
Δ WC (cm)	−3.4 ± 0.3*	−2.6 ± 0.2	−4.9 ± 0.6*	−3.2 ± 0.3	−5.8 ± 0.6*	−3.8 ± 0.4
Δ ALT (U/L)	−14.3 ± 1.4	−17.1 ± 1.3	−18.5 ± 2.4	−18.5 ± 1.6	−22.0 ± 3.0	−19.4 ± 1.8
ALT Normalization (%)	18.1*	7.1	32.5*	13.9	34.7*	19.9
Normal ALT (%)	31.8*	16.7	43.1*	22.4	45.8*	28.9
Δ GGT (U/L)	−7.4 ± 2.2*	−12.6 ± 1.7	−16.4 ± 5.2	−15.2 ± 2.0	−23.5 ± 6.9*	−16.1 ± 2.2
Δ TG (mg/dl)	−15.6 ± 5.6	−23.5 ± 4.0	−24.9 ± 7.8	−27.9 ± 7.9	−23.4 ± 9.8	−26.6 ± 5.1
Δ BG (mg/dl)	−4.3 ± 1.2	−7.9 ± 1.3	−5.9 ± 1.6	−9.6 ± 1.4	−5.7 ± 1.8	−9.0 ± 1.8

The percentage of cases who reached the desired 10% weight loss and ALT levels within the updated reference values is also reported (%). The total percentage of cases with normal ALT at each time point is also given.
ALT, alanine aminotransferase; BG, blood glucose; GGT, gamma-glutamyltransferase; TG, triglycerides; WC, waist circumference.
*
Significantly different from the corresponding value in Group-treated cases (chi-square, Fisher's exact, Mann-Whitney or Student's *t* test, as appropriate).

Weight loss >10% initial body weight was registered in 5%, 13% and 20% of cases in WBI at 6-, 12- and 24-month follow-up, and in 7%, 11% and 15% in GBI (all, *p* not significant). Another 20–28% of cases attained a weight loss >5% of initial body weight at different time points (Table 3). Weight cycling was not common; only 14 patients in GBI and 2 in WBI who had attained the 10% weight loss at 6- or 12-month follow-up regained weight and were no longer at target after two years (Fisher's exact test, $p = 0.166$) (Table 3).

Secondary outcomes

Changes in ALT levels and return within normal values

All liver enzymes decreased significantly in the course of the observation period, irrespective of treatment group (Fig. 2). This was mainly the case of ALT levels that declined on average by 22 ± SD 32 mU/ml in the two-year follow-up (Table 3).

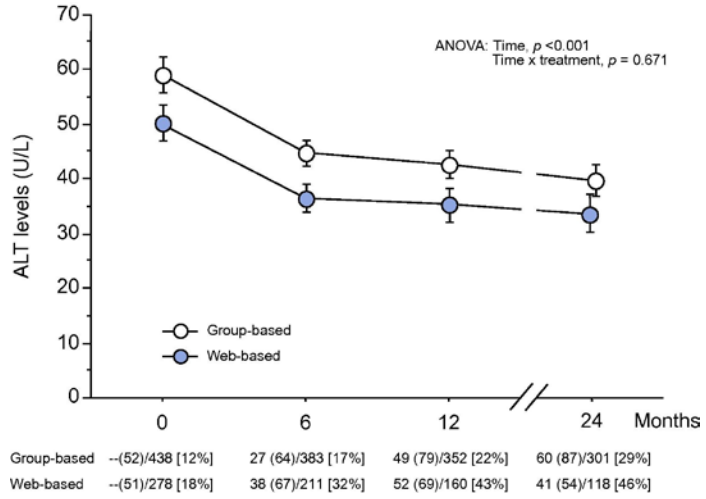


Fig. 2. **Time course of ALT levels in patients with NAFLD enrolled in the web-based (grey circles) and the group-based (white circles) lifestyle intervention programs.** In the Table, the numerator gives the number of cases with ALT decreased within normal range following treatment (the total number with normal ALT is in brackets), the denominator is the total number of cases, with the total percentage with normal ALT at different time points in square brackets. ALT, alanine aminotransferase; NAFLD, non-alcoholic fatty liver disease.

Overall, a higher number of cases had normal ALT levels in the course of follow-up. Among individuals with altered ALT levels at baseline, ALT normalized in 18% of cases after 6 months, in 32% at 12-month and 35% at 24-month follow-up in the WBI cohort. The corresponding values in the GBI group were 16% (vs. WBI, $p < 0.001$), 22% (vs. WBI, $p < 0.001$), and 29% (vs. WBI, $p = 0.002$) (Table 3).

Changes in surrogate markers of steatosis

The score of FLI decreased significantly in the course of the observation period in both groups (Fig. 3), more markedly in the WBI cohort after one (71.3 ± 20.9 vs. 78.0 ± 17.9 in GBI; p <0.001) and two years (68.9 ± 23.2 vs. 76.3 ± 19.2; p = 0.002). At 24 months FLI excluded steatosis (FLI <30) in 7% of WBI cases, 26% were classified as indeterminate (30<FLI<60), and steatosis was still diagnosed only in 67% of cases. The corresponding figures in GBI group were 3%, 19% and 78% (p = 0.053).

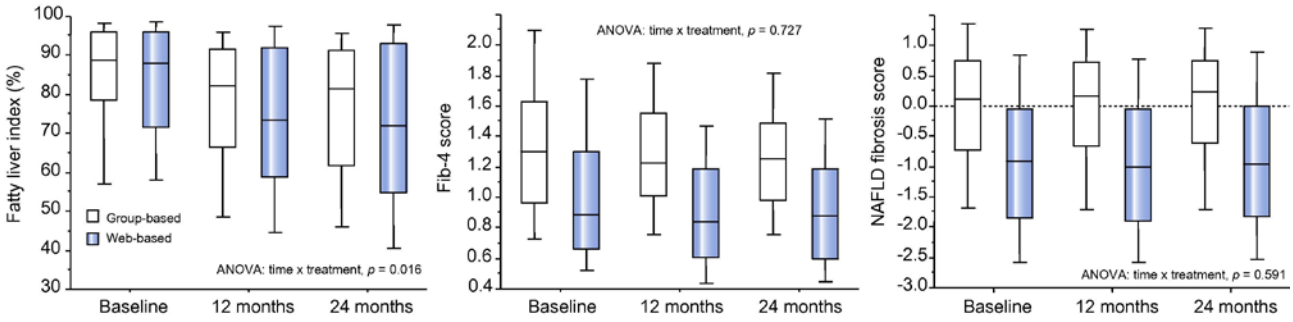


Fig. 3. Box plot representation of the time course of surrogate markers of steatosis and fibrosis in individuals enrolled in the group-based (white boxes) and web-based (grey boxes) lifestyle intervention programs. For all surrogate markers, ANOVA reveals significant differences between treatment categories (p <0.001).

Changes in Fib-4 and NAFLD fibrosis score

Both Fib-4 and NFS marginally decreased in the course of follow-up, and changes were significant for Fib-4 in both groups (Fig. 3). Severe fibrosis was no longer diagnosed by Fib-4 in four out of five cases available at follow-up in the control group, whereas no changes were observed in the web cohort. No significant changes in fibrosis score and stage were demonstrated by NFS.

Association of treatment and target reach

In logistic regression analysis, 10% weight loss after two years was only associated with baseline BMI (OR 1.43; 95% CI 1.13–1.81 per BMI/5). After adjustment for confounders (age, sex, education, employment, presence of diabetes, baseline BMI), the participation in WBI program did not significantly reduce the possibility of reaching the predefined weight loss target in comparison to GBI (Fig. 4). Additionally, assuming that all cases missed at follow-up did not attain the 10% weight loss at two years, this outcome was reached by intention-to-treat in 8.6% in WBI and 10.0% in GBI (p = 0.601), and participation in GBI did not reduce the probability of reaching the fully adjusted long-term 10% weight loss (OR 0.70; 95% CI 0.38–1.27).

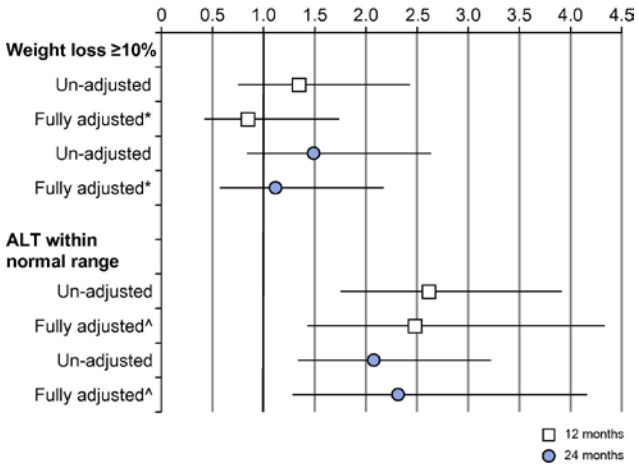


Fig. 4. Association of web-based lifestyle intervention with weight loss ≥10% initial body weight and ALT levels within normal values, compared with group-based lifestyle intervention, at 12-month (closed squares) and 24-month follow-up (closed circles). *Adjusted for age, sex, education, employment status, baseline BMI and ALT, and the presence of T2DM. ^Adjusted as above + weight change from baseline. ALT, alanine aminotransferase; BMI, body mass index; T2DM, type 2 diabetes mellitus.

WBI increased the rate of ALT normalization non-significantly at 6 months (OR 1.65; 95% CI 0.82–3.30), and significantly at 12 months (OR 2.58; 95% CI 1.41–4.71) and 24 months (OR 2.21; 95% CI 1.17–4.15), after adjustment for age, gender, education level, employment status, BMI and ALT at baseline, BMI changes at different time points, and the presence of T2DM (Fig. 4). Additional adjustment for calorie intake and physical activity at baseline did not change the results.

Discussion

The study demonstrates that web-based education is able to produce beneficial effects in patients with NAFLD, similar to those that can be obtained by group education. Although a higher number of cases were lost to follow-up in the web program, the total number who achieved the desired 10% weight loss target on intention-to-treat was similar, and it was also similar to the number who attained the same target in the Cuban experience.[12] This target was specifically chosen considering that it was associated with nearly universal NASH resolution at one-year follow-up liver biopsy, with improvement in steatosis, lobular inflammation and ballooning, and with stabilized or regressed fibrosis.[12] Notably, in our study the 10% weight loss was maintained at two years, accompanied by stable or improved surrogate markers of steatosis and fibrosis.

The use of web education in the management of non-communicable diseases has long been suggested, considering the huge number of cases at risk and patients' needs. The majority of cases are in an age range where job constraints make it difficult to implement a systematic face-to-face or group approach, whereas the eHealth procedures may keep the contact between patients and therapists without disrupting normal daily living.

The number of internet-based approaches for lifestyle changes in self-management programs has increased in several settings, both in adolescents and in adults,[25-27] and in different non-communicable diseases.[28-32] The most recent approaches allow for interaction with therapists, and also with peers in discrete groups, while maintaining privacy. Accordingly, they tend to overcome the drawbacks of classical approaches, based on face-to-face or group-based meetings with counselors, reaching the limited audience able to attend the clinic during working hours, in countries where treatment is regulated by a universal healthcare system, or are able to pay for private treatment.

In general, internet-based programs were shown to be less effective than face-to-face programs; their effectiveness may be increased by telephone recall systems[33] or text messages[34] but the reduced effectiveness is largely repaid by reduced costs and a larger audience.[35] This was also demonstrated in our setting, where nearly 60% of cases engaged in WBI lived outside the metropolitan area (and a few were scattered all over Italy), compared with the 27% of cases who entered the group-based behavioral approach.

The critical point in behavioral programs is engagement. Whenever Internet programs are used without any structured motivational approach, the results are expected to be poor. For this reason, the entry in our web program was regulated with the very same rules of access to the GBI program, *i.e.* the strategies of motivational interviewing according to Miller and Rollnick.[16] This strategy is the basis to achieve a significant adherence to the program[36] and has been successfully incorporated into behavioral programs aimed at healthy diet and habitual physical activity for weight loss in several diseases.[37-40] Patients were provided the user-id and password to access the system following a structured face-to-face meeting, and motivation to change was tested by appropriate, validated tests.[14] Reinforcements were provided during follow-up visits, in a similar way irrespective of treatment group.

Attrition was large in our population, and larger in the web-based cohort. A *post hoc* analysis of attrition rates showed that it was similar between those living within and outside the metropolitan area of Bologna (58% and 59%, respectively). Attrition remains a critical issue in the treatment of non-communicable diseases, where patients' behavior is pivotal to achieving favorable outcomes. Very high attrition rates (up to 50%) are also reported in recent industry-sponsored randomized controlled trials (RCTs) of pharmacological treatment of obesity.[41-43] where retention may be enhanced by the possibility of having treatment for free. The two-year follow-up rate measured in the present setting was much lower than usually found in the real world of obesity treatment,[44] where drop-out was not systematically associated with treatment failure.[45] In addition, attrition rates were associated with the presence of normal liver enzymes, in line with patients' belief that normal enzymes exclude progressive disease. Unfortunately, any additional contact with drop-outs is now precluded by Italian regulation on privacy, and no inference can be made on data in individuals missed at follow-up.

Both GBI and WBI interventions were effective in modifying dietary intake and physical activity, as demonstrated at six-month follow-up. Changes in calorie intake (approximately 200 kcal/day) are in agreement with the resulting two-year weight loss, and the negative energy balance was enlarged by small, but significant increases in physical activity in patients enrolled in WBI. Differences between cohorts are probably the result of differences in age and gender composition, which might also produce a different compliance to calorie restriction and physical activity, and should not be taken as proof of superiority of WBI *vs.* GBI. These differences should however be considered when planning lifestyle programs in free-living individuals with NAFLD.

Weight loss was accompanied by a remarkable reduction in liver enzymes, as well as stability or improvement in surrogate markers of steatosis and fibrosis. NASH and disease progression may also be present in individuals with normal aminotransferase levels,[46] but histological improvement is consistently associated with decreased aminotransferase, also in individuals with levels within the normal range at baseline. Normalization in the web-based cohort was not less common than observed in the group cohort, confirming that web education was at least as effective as the traditional approach. This conclusion is largely supported by the longitudinal analysis of surrogate markers, confirming a progressive reduction of steatosis with no worsening or improvement of fibrosis in individuals attending follow-up, without significant differences between cohorts.

The study has both strengths and limitations, which must be adequately discussed. The strengths are the novelty of the behavioral approach in this setting, the very large sample size, and a follow-up longer than that reported in randomized studies of behavioral treatment. Limitations are inherent in the observational nature of the study, with a control group reflecting the standard of care in NAFLD, as derived from clinical practice guidelines.[6]

Firstly, the general population might be partly different from the one usually tested in NAFLD RCTs. Individuals were recruited inside a unit serving as a specialist unit for NAFLD, but also caring a general population with obesity and diabetes. Accordingly, the BMI of patients might have been higher than usually observed in liver units, and the prevalence of T2DM might have been larger. However, in the most recent large RCTs published the mean BMI and the prevalence of T2DM were 35 kg/m^2 and 53% (obeticholic acid, FLINT study),[47] 31 kg/m^2 and <40% (elafibranor, GOLDEN-505 study)[48] and 34 kg/m^2 and 50% (cenicriviroc, CENTAUR study),[49] respectively. Accordingly, differences exist when compared with the NAFLD population principally enrolled in the U.S., but our cohort appears to be truly representative of the European NAFLD population. Notably, the population is very similar to that treated by lifestyle modifications in the behavioral intervention trial of Vilar-Gomez et al.[12]

Secondly, the population is scarcely defined by histology, and the effects of treatment are exclusively based on surrogate markers. Liver biopsy at baseline was available in less than 10% of cases, more commonly in patients entering the web-based cohort to be observed for a second opinion after being diagnosed in other centers. Liver disease was generally not severe, with a very limited proportion of cases with F3-4 fibrosis, and no statistically significant changes were

expected. The number of cases who achieved the weight loss target able to promote fibrosis regression was similar to that observed in the Cuban intervention, where only 11% of cases had fibrosis F3.[12] This suggests that the effectiveness of WBI, also in terms of histologic targets, might be comparable to that reported in the Cuban experience, but this remains to be demonstrated. Unfortunately, transient elastography was rarely performed (more commonly, in the web cohort and outside our institution, where it was not available when the project started), and the number of cases available at follow-up was insufficient for any analysis. Surrogate imaging techniques, now largely available in research hospitals might be used to confirm these beneficial effects.
Finally, the population enrolled into the internet program differed from the GBI population for a few, but important characteristics, including diabetes prevalence and visceral obesity. Although the association with target reach was tested after adjustment for confounders, the possibility remains that other features, not tested in the analysis, might have produced a confounding effect. Differences in age, education and job placement might have generated a stronger motivation to lifestyle changes not documented in the comparison with the GBI cohort. We would like to remark that the intent of including a GBI cohort in the report was not to demonstrate any superiority of a specific treatment approach, but merely to report the benchmark of the standard lifestyle approach used to derive WBI. Validation by properly designed randomized studies is needed for a proper comparison of the different lifestyle interventions.
In conclusion, this observational study indicates that an internet-based cognitive and educational program may be used to deliver multiple-session education to motivated patients with NAFLD who cannot attend face-to-face or group-based procedures. This approach produces beneficial behavioral changes, similar to those observed with the intensive lifestyle interventions carried out by trained teams, when added to motivational interviewing and counseling in a specific population, younger, less sick, technologically smart, maintained on a pre-programmed outpatient visit schedule. The WBI program might be extended to other units and/or general practitioners, increasing its impact in the community in prevention and treatment of progressive NAFLD. It might also be superimposed to drug treatment in the most severe cases, with possible additive effects. A likely opportunity is adding intensive lifestyle treatment to glucose-lowering therapy with glucagon-like peptide-1 receptor agonists, under investigation following pilot studies with positive results in NAFLD, at least in the population with prediabetes/diabetes.[50] Considering the burden of NAFLD in the community,[4] any large-scale strategy should be tested to reduce the burden of disease on patients and on healthcare systems.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Authors' contributions

Concept and design: GM, SDD; Patients care: AM, MTC, LB, GM; Writing: GM; Discussion and revision: AM, MTC, LB, MLF, EB, SP, GB; Statistical analysis: GM, GB. All authors approved the final version.

References

[1] Z.M. Younossi, A.B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, M. Wymer**Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes.** Hepatology, 64 (2016), pp. 73-84

[2] G. Marchesini, E. Bugianesi, G. Forlani, F. Cerrelli, M. Lenzi, R. Manini, *et al.***Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome.** Hepatology, 37 (2003), pp. 917-923

[3] G. Vernon, A. Baranova, Z.M. Younossi**Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults.** Aliment Pharmacol Ther, 34 (2011), pp. 274-285

[4] Z.M. Younossi, D. Blissett, R. Blissett, L. Henry, M. Stepanova, Y. Younossi, *et al.***The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe.** Hepatology, 64 (2016), pp. 1577-1586

[5] S.A. Townsend, P.N. Newsome**Review article: new treatments in non-alcoholic fatty liver disease.** Aliment Pharmacol Ther, 46 (2017), pp. 494-507

[6] European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity**EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease.** J Hepatol, 64 (2016), pp. 1388-1402

[7] M.L. Petroni, M.T. Caletti, S. Calugi, R. Dalle Grave, G. Marchesini**Long-term treatment of severe obesity: are lifestyle interventions still an option?** Exp Rev Endocrinol Metab, 12 (2017), pp. 391-400

[8] S. Moscatiello, R. Di Luzio, E. Bugianesi, A. Suppini, I. Hickman, S. Di Domizio, *et al.***Cognitive-behavioral treatment of non-alcoholic fatty liver disease: a propensity score-adjusted observational study.** Obesity (Silver Spring), 19 (2011), pp. 763-770

[9] K. Promrat, D.E. Kleiner, H.M. Niemeier, E. Jackvony, M. Kearns, J.R. Wands, *et al.***Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis.** Hepatology, 51 (2010), pp. 121-129

[10] C. Thoma, C.P. Day, M.I. Trenell**Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review.** J Hepatol, 56 (2012), pp. 255-266

[11] V.W. Wong, R.S. Chan, G.L. Wong, B.H. Cheung, W.C. Chu, D.K. Yeung, *et al.***Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial.** J Hepatol, 59 (2013), pp. 536-542

[12] E. Vilar-Gomez, Y. Martinez-Perez, L. Calzadilla-Bertot, A. Torres-Gonzalez, B. Gra-Oramas, L. Gonzalez-Fabian, *et al.***Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis.** Gastroenterology, 149 (2015), pp. 367-378 e5; quiz e14–5

[13] G. Marchesini, S. Petta, R. Dalle Grave**Diet, weight loss, and liver health in nonalcoholic fatty liver disease: pathophysiology, evidence, and practice.** Hepatology, 63 (2016), pp. 2032-2043

[14] E. Centis, S. Moscatiello, E. Bugianesi, S. Bellentani, A.L. Fracanzani, S. Calugi, *et al.***Stage of change and motivation to healthier lifestyle in non-alcoholic fatty liver disease.** J Hepatol, 58 (2013), pp. 771-777

[15] K. Kempf, B. Altpeter, J. Berger, O. Reuss, M. Fuchs, M. Schneider, *et al.***Efficacy of the telemedical lifestyle intervention program TeLiPro in advanced stages of type 2 diabetes: a randomized controlled trial.** Diabetes Care, 40 (2017), pp. 863-871

[16] W.R. Miller, S. Rollnick**Motivational Interviewing.** (2nd ed.), The Guilford Press, New York (2002)

[17] G. Tarrini, S. Di Domizio, R. Rossini, A. Romano, F. Cerrelli, G. Marchesini, *et al.***Quanto mangio veramente?** G Ital Diabetol Metab, 26 (2006), pp. 48-53

[18] R. Rossini, S. Moscatiello, G. Tarrini, S. Di Domizio, V. Soverini, A. Romano, *et al.***Effects of cognitive-behavioral treatment for weight loss in family members.** J Am Diet Assoc, 111 (2011), pp. 1712-1719

[19] C.L. Craig, A.L. Marshall, M. Sjostrom, A.E. Bauman, M.L. Booth, B.E. Ainsworth, *et al.***International physical activity questionnaire: 12-country reliability and validity.** Med Sci Sports Exerc, 35 (2003), pp. 1381-1395

[20] G. Forlani, C. Lorusso, S. Moscatiello, V. Ridolfi, N. Melchionda, S. Di Domizio, *et al.***Are behavioural approaches feasible and effective in the treatment of type 2 diabetes? A propensity score analysis vs. prescriptive diet.** Nutr Metab Cardiovasc Dis, 19 (2009), pp. 313-320

[21] G. Bedogni, S. Bellentani, L. Miglioli, F. Masutti, M. Passalacqua, A. Castiglione, *et al.***The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population.** BMC Gastroenterol, 6 (2006), p. 33

[22] P. Angulo, J.M. Hui, G. Marchesini, E. Bugianesi, J. George, G.C. Farrell, *et al.***The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD.** Hepatology, 45 (2007), pp. 846-854

[23] A. Vallet-Pichard, V. Mallet, B. Nalpas, V. Verkarre, A. Nalpas, V. Dhalluin-Venier, *et al.***FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest.** Hepatology, 46 (2007), pp. 32-36

[24] N. Melchionda, G. Forlani, L. La Rovere, P. Argnani, F. Trevisani, D. Zocchi, *et al.***Disease management of the metabolic syndrome in a community: study design and process analysis on baseline data.** Metab Syndr Relat Disord, 4 (2006), pp. 7-16

[25] K.M. McTigue, M.B. Conroy, R. Hess, C.L. Bryce, A.B. Fiorillo, G.S. Fischer, *et al.***Using the internet to translate an evidence-based lifestyle intervention into practice.** Telemed J E Health, 15 (2009), pp. 851-858

[26] T.J. Moore, N. Alsabeeh, C.M. Apovian, M.C. Murphy, G.A. Coffman, D. Cullum-Dugan, *et al.***Weight, blood pressure, and dietary benefits after 12 months of a Web-based Nutrition Education Program (DASH for health): longitudinal observational study.** J Med Internet Res, 10 (2008), p. e52

[27] P. Sousa, H. Fonseca, P. Gaspar, F. Gaspar**Controlled trial of an Internet-based intervention for overweight teens (Next.Step): effectiveness analysis.** Eur J Pediatr, 174 (2015), pp. 1143-1157

[28] R.M. Banos, M.S. Mensorio, A. Cebolla, E. Rodilla, G. Palomar, J. Lison, *et al.***An Internet-based self-administered intervention for promoting healthy habits and weight loss in hypertensive people who are overweight or obese: a randomized controlled trial.** BMC Cardiovasc Disord, 15 (2015), p. 83

[29] A.P. Cotter, N. Durant, A.A. Agne, A.L. Cherrington**Internet interventions to support lifestyle modification for diabetes management: a systematic review of the evidence.** J Diabetes Complications, 28 (2014), pp. 243-251

[30] S. Kodama, K. Saito, S. Tanaka, C. Horikawa, K. Fujiwara, R. Hirasawa, *et al.***Effect of Web-based lifestyle modification on weight control: a meta-analysis.** Int J Obes (Lond), 36 (2012), pp. 675-685

[31] K.M. Livingstone, C. Celis-Morales, S. Navas-Carretero, R. San-Cristobal, A.L. Macready, R. Fallaize, *et al.***Effect of an Internet-based, personalized nutrition randomized trial on dietary changes associated with the Mediterranean diet: the Food4Me Study.** Am J Clin Nutr, 104 (2016), pp. 288-297

[32] D. Sherifali, R. Hess, K.M. McTigue, A. Brozic, K. Ng, H. Gerstein**Evaluating the feasibility and impact of an internet-based lifestyle management program in a diabetes care setting.** Diabetes Technol Ther, 16 (2014), pp. 358-362

[33] R. Eagleson, L. Altamirano-Diaz, A. McInnis, E. Welisch, S. De Jesus, H. Prapavessis, *et al.***Implementation of clinical research trials using web-based and mobile devices: challenges and solutions.** BMC Med Res Methodol, 17 (2017), p. 43

[34] A.L. Fortmann, L.C. Gallo, M.I. Garcia, M. Taleb, J.A. Euyoque, T. Clark, *et al.***Dulce digital: an mHealth SMS-based intervention improves glycemic control in hispanics with type 2 diabetes.** Diabetes Care, 40 (2017), pp. 1349-1355

[35] R.S. Rasu, C.M. Hunter, A.L. Peterson, H.M. Maruska, J.P. Foreyt**Economic evaluation of an Internet-based weight management program.** Am J Manag Care, 16 (2010), pp. e98-e104

[36] R. Dalle Grave, E. Centis, R. Marzocchi, M. El Ghoch, G. Marchesini**Major factors for facilitating change in behavioral strategies to reduce obesity.** Psychol Res Behav Manag, 6 (2013), pp. 101-110

[37] M.J. Armstrong, T.A. Mottershead, P.E. Ronksley, R.J. Sigal, T.S. Campbell, B.R. Hemmelgarn**Motivational interviewing to improve weight loss in overweight and/or obese patients: a systematic review and meta-analysis of randomized controlled trials.** Obes Rev, 12 (2011), pp. 709-723

[38] P.D. O'Halloran, F. Blackstock, N. Shields, A. Holland, R. Iles, M. Kingsley, *et al.***Motivational interviewing to increase physical activity in people with chronic health conditions: a systematic review and meta-analysis.** Clin Rehabil, 28 (2014), pp. 1159-1171

[39] S.A. Simpson, R. McNamara, C. Shaw, M. Kelson, Y. Moriarty, E. Randell, *et al.***A feasibility randomised controlled trial of a motivational interviewing-based intervention for weight loss maintenance in adults.** Health Technol Assess, 19 (2015) v-vi, xix-xxv, 1–378

[40] D. Smith West, V. DiLillo, Z. Bursac, S.A. Gore, P.G. Greene**Motivational interviewing improves weight loss in women with type 2 diabetes.** Diabetes Care, 30 (2007), pp. 1081-1087

[41] X. Pi-Sunyer, A. Astrup, K. Fujioka, F. Greenway, A. Halpern, M. Krempf, *et al.***A randomized, controlled trial of 3.0 mg of liraglutide in weight management.** N Engl J Med, 373 (2015), pp. 11-22

[42] S.E. Nissen, K.E. Wolski, L. Prcela, T. Wadden, J.B. Buse, G. Bakris, *et al.***Effect of naltrexone-bupropion on major adverse cardiovascular events in overweight and obese patients with cardiovascular risk factors: a randomized cinical trial.** JAMA, 315 (2016), pp. 990-1004

[43] K.M. Gadde, D.B. Allison, D.H. Ryan, C.A. Peterson, B. Troupin, M.L. Schwiers, *et al.***Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial.** Lancet, 377 (2011), pp. 1341-1352

[44] R. Dalle Grave, N. Melchionda, S. Calugi, E. Centis, A. Tufano, G. Fatati, *et al.***Continuous care in the treatment of obesity: an observational multicentre study.** J Intern Med, 258 (2005), pp. 265-273

[45] E. Grossi, R. Dalle Grave, E. Mannucci, E. Molinari, A. Compare, M. Cuzzolaro, *et al.***Complexity of attrition in the treatment of obesity: clues from a structured telephone interview.** Int J Obes (Lond), 30 (2006), pp. 1132-1137

[46] A.L. Fracanzani, L. Valenti, E. Bugianesi, M. Andreoletti, A. Colli, E. Vanni, *et al.***Risk of severe liver disease in NAFLD with normal aminotransferase levels: a role for insulin resistance and diabetes.** Hepatology, 48 (2008), pp. 792-798

[47] B.A. Neuschwander-Tetri, R. Loomba, A.J. Sanyal, J.E. Lavine, M.L. Van Natta, M.F. Abdelmalek, *et al.***Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial.** Lancet, 385 (2015), pp. 956-965

[48] V. Ratziu, S.A. Harrison, S. Francque, P. Bedossa, P. Lehert, L. Serfaty, *et al.***Elafibranor, an agonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening.** Gastroenterology, 150 (2016), p. e5

[49] S.L. Friedman, V. Ratziu, S.A. Harrison, M.F. Abdelmalek, G.P. Aithal, J. Caballeria, *et al.***A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis.** Hepatology, 67 (2018), pp. 1754-1767

[50] M.J. Armstrong, P. Gaunt, G.P. Aithal, D. Barton, D. Hull, R. Parker, *et al.***Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study.** Lancet, 387 (2016), pp. 679-690